



The effect of magnesium supplementation on vascular calcification in chronic kidney disease-a randomised clinical trial (MAGiCAL-CKD)

essential study design and rationale

Bressendorff, Iain; Hansen, Ditte; Schou, Morten; Kragelund, Charlotte; Brandi, Lisbet

Published in:
B M J Open

DOI:
[10.1136/bmjopen-2017-016795](https://doi.org/10.1136/bmjopen-2017-016795)

Publication date:
2017

Document version
Publisher's PDF, also known as Version of record

Document license:
[CC BY-NC](#)

Citation for published version (APA):
Bressendorff, I., Hansen, D., Schou, M., Kragelund, C., & Brandi, L. (2017). The effect of magnesium supplementation on vascular calcification in chronic kidney disease-a randomised clinical trial (MAGiCAL-CKD): essential study design and rationale. *B M J Open*, 7(6), [e016795]. <https://doi.org/10.1136/bmjopen-2017-016795>

BMJ Open The effect of magnesium supplementation on vascular calcification in chronic kidney disease – a randomised clinical trial (MAGiCAL-CKD): essential study design and rationale

Iain Bressendorff,¹ Ditte Hansen,² Morten Schou,³ Charlotte Kragelund,³ Lisbet Brandt¹

To cite: Bressendorff I, Hansen D, Schou M, *et al*. The effect of magnesium supplementation on vascular calcification in chronic kidney disease—a randomised clinical trial (MAGiCAL-CKD): essential study design and rationale. *BMJ Open* 2017;7:e016795. doi:10.1136/bmjopen-2017-016795

► Prepublication history for this paper is available online. To view these files please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2017-016795>).

Received 10 March 2017
Revised 26 April 2017
Accepted 2 May 2017



CrossMark

¹Department of Cardiology, Nephrology, and Endocrinology, Nordsjællands Hospital, Hillerød, Denmark

²Department of Nephrology, Herlev and Gentofte Hospital, Herlev, Denmark

³Department of Cardiology, Herlev and Gentofte Hospital, Herlev, Denmark

Correspondence to

Dr Iain Bressendorff;
iaib@bressendorff.com

ABSTRACT

Introduction Chronic kidney disease (CKD) is associated with an increased risk of cardiovascular disease and mortality, which is thought to be caused by increased propensity towards vascular calcification (VC). Magnesium (Mg) inhibits phosphate-induced VC in vitro and in animal models and serum Mg is inversely associated with cardiovascular mortality in predialysis CKD and in end-stage renal disease. This paper will describe the design and rationale of a randomised double-blinded placebo-controlled multicentre clinical trial, which will investigate whether oral Mg supplementation can prevent the progression of coronary artery calcification (CAC) in subjects with predialysis CKD.

Methods and analysis We will randomise 250 subjects with estimated glomerular filtration rate of 15 to 45 mL/min/1.73 m² to 12 months treatment with either slow-release Mg hydroxide 30 mmol/day or matching placebo in a 1:1 ratio. The primary end point is change in CAC score as measured by CT at baseline and after 12 months treatment. Secondary end points include change in pulse wave velocity, bone mineral density, measures of mineral metabolism and clinical end points related to cardiovascular and renal events.

Ethics and dissemination This trial has been approved by the local biomedical research ethics committees and data protection agencies and will be performed in accordance with the latest revision of the Helsinki Declaration. The trial will examine for the first time the effect of increasing the uptake of a putative VC inhibitor (ie, Mg) on progression of CAC in subjects with predialysis CKD.

Trial registration number NCT02542319, pre-results.

INTRODUCTION

Chronic kidney disease (CKD) is prevalent in approximately 13% of the general population¹ and is associated with an increased risk of developing cardiovascular disease (CVD).² This is partly due to an accumulation of

Strengths and limitations of this study

- Randomised, double-blind, placebo-controlled study design.
- Multicentre trial.
- Sufficient sample size and follow-up.
- Novel intervention.
- Surrogate end points, no hard clinical endpoints, for example, cardiovascular events or mortality.
- Unknown whether results can be extrapolated to more severe forms of kidney disease.

traditional CVD risk factors (eg, hypertension, dyslipidaemia, diabetes mellitus (DM), age), but even after adjusting for these risk factors, CKD is still associated with an increased risk of CVD as compared with the general population.² This implies that other non-traditional CVD risk factors associated with CKD are causing an increased risk of CVD. Vascular calcification (VC) is highly prevalent among persons with CKD³ and is thought to be partly responsible for this increased risk of CVD. Indeed, indirect measurements of VC (eg, coronary artery calcifications score (CAC), pulse wave velocity (PWV) and pulse pressure) have been associated with increased risk of CVD (particularly heart failure) in persons with CKD.^{4–6} Thus, any intervention that could prevent the development or progression of VC is presumed also to reduce the risk of CVD and death.

In subjects with CKD, disturbances in the mineral and bone metabolism are thought to be one of the main causes of VC. As kidney function declines, phosphate (PO₄) accumulates in serum and binds to calcium (Ca). Once the concentration of Ca/PO₄ particles

in serum exceed their saturation point, they precipitate and induce the vascular smooth muscle cells (VSMC) of the arteries to undergo a dedifferentiation into an osteogenic phenotype in which the VSMC calcify and stiffen.⁷ So far, efforts to prevent VC in CKD have been focused on the concentration of calcification promoters in serum (ie, PO_4 and Ca), but recently focus has shifted to also consider the concentration of calcification inhibitors in serum. *In vitro* studies of PO_4 -induced VC and animal studies of CKD and VC have consistently shown that increasing magnesium (Mg) levels prevents or reduces the development of VC^{8–15} by increasing the expression of intracellular calcification inhibitors and decreasing the expression of calcification promoters and PO_4 transporters. Several epidemiological studies have shown that higher levels of serum Mg are associated with improved survival in patients with CKD¹⁶ and end-stage renal disease (ESRD).^{17–24} Also, higher levels of serum Mg are associated with reduced incidence and progression of CKD^{25–27} and with lower prevalence of VC in CKD.²⁸

In this setting, increasing the Mg concentration in serum by means of Mg supplementation is an attractive prospect. Two small clinical trials of Mg supplementation in ESRD have shown that this intervention can reduce the progression of carotid intima/media thickness (a proxy for VC) with no incidences of toxic hypermagnesaemia.^{29–30} So far, no clinical trials have investigated the effect of Mg supplementation on VC in predialysis CKD. We therefore posed the research question of whether Mg supplementation is effective in preventing the progression of VC in CKD.

In this paper, we will describe the design and rationale of our randomised placebo-controlled double-blinded multicentre clinical trial designed to investigate whether 52 weeks of treatment with oral Mg supplementation can prevent the progression of CAC in subjects with CKD stage 3b–4.

METHODS AND ANALYSIS

Study design and population

This trial is an investigator-initiated double-blind placebo-controlled multicentre clinical trial being performed at 10 sites in departments of nephrology in Denmark and Norway. We will include adult persons with CKD stage 3b–4 whom we consider to be at increased risk of VC based on serum Mg and PO_4 . The full list of inclusion and exclusion criteria is shown in figure 1.

Study intervention

The subjects participating in the trial will be randomised in a ratio of 1:1 to 52 weeks treatment with either oral slow-release Mg hydroxide (Mablet 360mg, Gunnar Kjems, Denmark) twice daily or matching placebo twice daily (figure 2). Mablet is used for Mg supplementation and one tablet containing 360mg of Mg hydroxide is equivalent to 15mmol of elemental Mg, and the intervention will therefore consist of a daily dose of 30mmol of

elemental Mg. Mg hydroxide and placebo tablets are identical in appearance, odour, constituents and containers and do not contain calcium. The study medication has been packed in consecutively numbered containers according to a computer-generated randomisation list and will be administered to subjects consecutively as they enter the trial. The randomisation will be stratified based on enrolment site and DM. Both subjects and investigators will be blinded to the study medication during the course of the trial.

Study visits

All persons being followed in the outpatient clinics at the participating sites will be screened for eligibility prior to their planned appointments in the clinics and offered participation in the trial.

At week 0, subjects will give written informed consent to participate in the trial, and will have their relevant medical history recorded along with anthropomorphic measurements. After this, they will undergo a multislice ECG gated CT scan of the coronary arteries of their heart to calculate the extent of any VC based on CAC by the Agatston method.³¹ In addition, subjects will have fasting blood samples drawn for assessment of kidney function, electrolytes, mineral metabolism and serum calcification propensity (T_{50}).³² Samples from a 24-hour urine collection will be collected to assess urinary excretion of minerals and protein, and a resting ECG will be performed to assess length of the PQ interval and the corrected QT interval (QT_c). At selected sites, subjects will also undergo measurement of bone mineral density (BMD) of their lumbar vertebrae and hip during their CT scan, and arterial tonometry will be performed to assess carotid-femoral PWV (CF-PWV) and radial PWA (see figure 3 for the full list of assessments at study visits).

Following the baseline visit, each subject will be seen for follow-up visits at 13-week intervals (ie, week 13, week 26 and week 39), at which times subjects will have fasting blood samples drawn to assess safety of the intervention (see the Safety monitoring section), 24 hours urine collections will be sampled to assess mineral excretion and study medication will be dispensed. Adherence to the intervention will be assessed by pill count of any remaining study medication from the previous study visit.

At the final study visit (week 52), subjects will repeat baseline measurements, after which they will return to their regular outpatient care.

Safety monitoring

All subjects will have serum Mg and potassium evaluated at each follow-up visit and will be questioned on any potential side effects to the treatment, particularly gastrointestinal side effects such as loosening of stool or diarrhoea. If at any time serum Mg exceeds 1.80mmol/L, the subject will cease study medication, and a follow-up blood sample for serum Mg will be drawn 2 weeks later. If at this time serum Mg still exceeds 1.50mmol/L, the subject will permanently cease study medication, but will

Inclusion criteria

1. Age \geq 18 years.
2. Estimated glomerular filtration rate between 15 and 45 mL/min for > 3 months.
3. Considered at risk of developing vascular calcification. Defined as one of the following:
 - 3.1 Serum Mg < 0.82 mmol/L and serum PO₄ > 1.15 mmol/L on average of previous measurements.
 - 3.2 Serum Mg < 0.92 mmol/L and serum PO₄ > 1.30 mmol/L on average of previous measurements.
4. Life expectancy > 12 months.
5. Expected time until initiation of dialysis or kidney transplantation > 12 months.
6. Women of childbearing age must be actively using reliable contraceptive therapy. Defined as:
 - 6.1 Hormonal contraceptive (oral, cervical ring, injection, transdermal patch).
 - 6.2 Intrauterine device.
7. A negative serum pregnancy test.
8. Written informed consent.

Exclusion criteria

1. Current treatment with haemodialysis or peritoneal dialysis.
2. Kidney donor recipient.
3. Previous coronary artery bypass graft.
4. Parathyroid hormone > 66 pmol/L.
5. Previous parathyroidectomy.
6. Current treatment with Mg containing medication or supplements.
7. Any condition impairing Mg absorption from the gastrointestinal tract (e.g. short bowel syndrome, chronic pancreatitis).
8. Active malignancy (basal or squamous cell skin carcinoma, localised prostate cancer and cancer with no signs of reoccurrence after five years are exempt from this).
9. Other diseases or conditions, which, in the opinion of the site investigator, would prevent participation in or completion of trial.
10. Pregnancy or breastfeeding.
11. Allergy towards contents of interventional medication.
12. Participation in other interventional trials.

Abbreviations:

Mg, magnesium; PO₄, phosphate.

Figure 1 Inclusion and exclusion criteria.

otherwise continue in the trial as planned. If serum Mg does not exceed 1.50 mmol/L the subject will continue study medication at half dose (ie, only take study medication once daily) and a follow-up blood sample for serum Mg will be drawn again 2 weeks later. If at this time serum Mg exceeds 1.50 mmol/L, the subject will permanently cease study medication, but will otherwise continue in the trial as planned. Any incidences of hyperkalaemia will be handled at the discretion of the local site investigator.

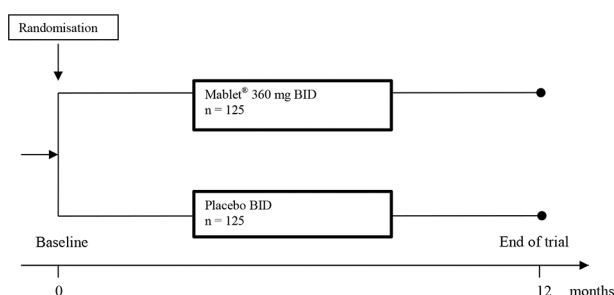


Figure 2 Trial design of effect of oral magnesium on vascular calcification in chronic kidney disease. BID, twice daily.



Adverse events and serious adverse events will be recorded and reported to an independent data and safety monitoring board.

Concomitant medical therapy

Subjects should not initiate treatment with Mg-containing medication, laxatives or antacids during the trial. Also, investigators should avoid initiating medical therapy that might reduce Mg absorption in the gut or Mg loss in the kidneys. Therefore, investigators should avoid initiating proton pump inhibitors unless necessary for serious gastrointestinal conditions (histamine H₂-receptor antagonist may be considered as an alternative), and if possible investigators should not initiate or increase doses of thiazide diuretics, thiazide-like diuretics or loop diuretics for treatment of hypertension and should instead use other antihypertensive agents. All medications and any changes to these will be recorded at baseline and at each follow-up visit.

Study end points

The primary end point of this trial is the difference in change of CAC score from week 0 to week 52 between

	Enrolment	Allocation	Post-allocation			Close-out
TIMEPOINT	Screening	Week 0	Week 13	Week 26	Week 39	Week 52
<u>ENROLMENT:</u>						
Eligibility screen	X					
Informed consent		X				
Allocation		X				
<u>INTERVENTION:</u>						
Mablet® 360 mg BID (n = 125)						
Placebo BID (n = 125)						
<u>ASSESSMENTS:</u>						
CAC by CT scan		X				X
BMD by CT scan*		X				X
CF-PWV and PWA*		X				X
Electrocardiogram		X				X
Fasting blood samples**		X	X	X	X	X
T ₅₀ , FGF23, biobank samples		X				X
24-hour urine collection***		X	X	X	X	X

* At selected sites only.

** Magnesium, phosphate, ionised calcium, parathyroid hormone, bicarbonate, albumin, creatinine, urea, sodium, potassium, C-reactive protein, haemoglobin, leucocytes, thrombocytes, haemoglobin A_{1C}.

*** Magnesium, phosphate, calcium, creatinine, protein.

Abbreviations:

BID, twice daily; BMD, bone mineral density; CAC, coronary artery calcification score; CF-PWV, carotid-femoral pulse wave velocity; CT, computer tomography; FGF23, fibroblast growth factor 23; PWA, pulse wave analysis; T₅₀, serum calcification propensity.

Figure 3 Schedule for enrolment, interventions and assessments.

the two groups. CAC scores will be calculated independently by two observers trained in CAC scoring. In the event of >5% difference in calculated CAC score, the score will be recalculated by both observers together for a final CAC score. Subgroup analyses will be performed to assess the effect of the intervention on CAC progression in subjects with and without DM, in subjects with CAC >0 and CAC=0 at baseline and in subjects divided into tertiles of T₅₀.

Secondary end points include change and CF-PWV and PWA, change in BMD, change in markers of mineral metabolism, difference in rate of estimated glomerular filtration rate (eGFR) decline, as well as

clinical end points such as incidence of major adverse cardiovascular events (myocardial infarction, stroke, new-onset heart failure or hospitalisation for heart failure and limb amputation or revascularisation due to peripheral arterial occlusion), all-cause and cardiovascular mortality, adverse and serious adverse events and incidence of ESRD (defined as dialysis initiation or kidney transplantation). In addition, subjects will be contacted 5 years after their participation in the study to assess whether Mg supplementation reduces the incidence of major adverse cardiovascular events, death or ESRD.

Statistical analysis

Data that follow a normal distribution will be described as mean \pm SD and non-normal data will be described as median with IQR. The primary end point will be compared between the two treatment groups using an unpaired Student's t-test (possibly after logarithmic transformation if the data do not follow a normal distribution) and as intention to treat. Changes and between-group differences in secondary end points will be analysed using paired and unpaired Student's t-tests or Wilcoxon and Mann-Whitney tests for continuous variables and χ^2 or Fisher's exact test for dichotomous variables. Linear mixed models will be used to analyse variables with repeated measures. Linear and logistic regression analyses with adjustment for various covariates will be applied to baseline data to investigate any associations with the change in various parameters as well as any associations with clinical events.

The data analysis will be performed blinded to the treatment allocation.

Sample size calculation

In a prospective observational study of 53 patients with CKD stage 3–5, 27 patients (51%) had CAC at baseline.³³ Of these 27 patients, the annual progression of CAC score was 275.6 \pm 69.2 (mean and SE of the mean). The SD at baseline for CAC score in this group of 27 patients was 360. We consider an annual difference in CAC score of 200 to be the minimally relevant difference, since a difference in change of this magnitude has been shown in a previous trial of phosphate binder treatment in CKD stage 3–5.³⁴ With a power (β) of 20% in a two-sample t-test with a significance (α) of 5% the sample size must be 52 per group, when considering only the data concerning the patients who had CAC at baseline. However, since we do not know whether subjects will have CAC or not at baseline, we must assume that the prevalence (51%) and progression of CAC in our population will be similar to the reference study. Based on this, we therefore need 108 subjects per group. We anticipate a dropout rate of 15%, and the trial will therefore include 250 subjects randomised in a ratio of 1:1.

Ethics and dissemination

All subjects will give written informed consent prior to initiating the trial and the trial will be performed according to the latest revision of the Helsinki Declaration. We applied for and received approval to conduct the trial from the Danish and Norwegian National Committees on Biomedical Research Ethics (H-15009846 and REK Sør-Øst D 2015/2428, respectively) as well as the Danish and Norwegian Data Protection Agencies (2012-58-0004 and 16-077, respectively). Since Mablet is registered as a supplement and not a drug, the trial does not require approval from the Danish or Norwegian medical agencies. We have established an independent data safety monitoring board at Nordsjællands Hospital, Hillerød, Denmark, which will monitor all serious adverse events

occurring during the trial. Lastly, we registered the trial at www.clinicaltrials.gov (NCT02542319) prior to initiating the trial.

We began recruitment in December 2015 and expect to have completed recruitment by September 2017 and completed data collection by September 2018. The final results of the trial will be published in international peer-reviewed journals.

DISCUSSION

Assessing risk of VC progression

Serum PO₄ is the only modifiable risk factor that has been associated with progression of CAC in predialysis CKD,³³ and all published in vitro and animal studies of Mg in PO₄-induced VC have shown that Mg ameliorates the development of VC.^{8–15} Thus, PO₄ likely promotes CAC progression in CKD, and increasing levels of Mg seem to prevent VC induced by PO₄. Therefore, it seems reasonable to consider patients with 'increased' PO₄ and 'reduced' levels of Mg to be at risk of CAC progression.

In a previous randomised clinical trial of CAC progression in patients with predialysis CKD and no history of coronary artery disease, previous stroke, DM or dyslipidaemia (ie, free of important conventional risk factors associated with VC and thus reflecting 'uremic' causes of VC), the placebo group had a mean serum PO₄ of 1.26 mmol/L at baseline and experienced an annual progression of CAC by approximately 200.³⁴ Unfortunately, no trials in predialysis CKD have examined the quantitative role of Mg in CAC progression. However, one cross-sectional study found that for PO₄ <1.10 mmol/L there was no relationship between serum Mg and CAC density, while for PO₄ >1.10 mmol/L there was an almost linear inverse relationship between serum Mg and CAC density.²⁸ Based on this, we have made the assumptions that serum PO₄ >1.10 mmol/L is necessary for serum Mg to exert its calcification inhibitory effects, and serum PO₄ >1.26 mmol/L is predictive of CAC progression when serum Mg is unknown. We have therefore semiarbitrarily set our threshold for risk of CAC progression at serum Mg <0.82 (ie, below the mean laboratory value of Mg) when serum PO₄ >1.15 mmol/L, or serum Mg <0.92 (ie, below the upper laboratory limit of Mg) when serum PO₄ >1.30 mmol/L.

We acknowledge that several other factors involved in mineralisation are likely involved in the propensity towards CAC, and that our assumption does not account for these. However, there is no available evidence linking these other factors with risk of CAC progression and most are not implemented in clinical practice. Therefore, we have chosen a pragmatic approach to assessment of VC risk based only on serum PO₄ and Mg.

Safety and efficacy of Mg supplementation

Prior to the design of this trial, we conducted a randomised double-blind placebo-controlled clinical trial to examine the safety and efficacy of 8 weeks of

slow-release Mg hydroxide supplementation in subjects with eGFR <60 mL/min/1.73 m² and low or low-normal serum Mg, and found that a dose of 30 mmol/day was necessary to achieve statistically significant increases in serum Mg.³⁵ This intervention was found to be safe (ie, no incidences of serum Mg>2.0 mmol/L) and the incidences of gastrointestinal side effects were similar to placebo treatment. Also, this intervention increased T₅₀, which is believed to reflect the propensity towards ectopic calcification and which has been shown to be inversely associated with all-cause mortality in a predialysis CKD.³⁶ The pilot trial was small and of only 8 weeks duration, so it is possible that there are side effects or toxicities related to the chosen dose of Mg supplementation, which will only become manifest after longer treatment periods.

We chose the slow-release Mg hydroxide formulation used in this trial because it is cheap and widely available in Denmark. Due to its slow-release formulation, it does not have the laxative effect usually associated with inorganic Mg compounds, although long-term use or high doses are known to cause loosening of stool and/or frequent bowel movements. It is possible that other organic Mg formulations (eg, Mg citrate) might have greater bioavailability of Mg^{37 38} and fewer gastrointestinal side effects, but to our knowledge no clinical trials have examined their safety and efficacy in subjects with CKD.

Mg supplementation as a PO₄ binder

Given the known PO₄-lowering effects of Mg-containing PO₄ binders,³⁹ it is possible that the Mg supplementation used in this trial might also reduce serum PO₄. We found no effect of Mg supplementation on serum PO₄, 24 hours urine PO₄ or intact fibroblast growth factor 23 in our previously mentioned trial of Mg supplementation, suggesting that the Mg formulation used in this trial does not act as a PO₄ binder.³⁵ The size and duration of this trial will make it possible to better assess any effects of Mg supplementation on PO₄ homeostasis, although any results must be interpreted with caution, since this trial is not designed to address this research question.

Limitations of this trial

The main limitation of this trial is that CAC is only a surrogate measure for CVD. Even if Mg supplementation is effective in reducing the progression of CAC, this trial is not powered to assess whether reduced progression of CAC reduces the incidence of cardiovascular events or mortality.

Although intimal and medial calcification often occur simultaneously, calcification due to disturbances in mineral and bone metabolism is believed mainly to cause medial calcification. CAC by the Agatston method measures the product of volume and density of calcium in the coronary arteries but cannot differentiate between intimal and medial calcification, and thus it is not possible to assess whether any changes to CAC are due to intimal calcification, medial calcification or both. Mg

supplementation might also reduce progression of aortic calcification and thereby progression of aortic stiffness, in which case CF-PWV might be affected by the intervention. CF-PWV will be measured in a subset of the subjects participating in the trial.

A further limitation of this trial is that the trial will only include subjects with CKD stage 3b-4. Patients with stage 5 CKD will likely have greater risk of VC progression and would therefore potentially have greater benefit of any treatment effect. However, since there are no clinical trials assessing the safety of Mg supplementation in stage 5 CKD, we have excluded patients with eGFR <15 mL/min/1.73 m² from this trial. It is likely that some subjects will progress to stage 5 CKD during the course of the trial, and if any of these subjects are randomised to Mg supplementation, we will learn more about the safety of Mg supplementation in subjects with very low eGFR.

Potential impact of the trial

Previous trials have examined the effect of PO₄ binders on progression of VC in predialysis CKD with varying results.^{34 40 41} This trial tests a new approach to the prevention of VC, by attempting to increase the uptake of a calcification inhibitor instead of limiting the uptake of a calcification promoter. If successful, this trial might pave the way for larger randomised clinical trials investigating the effects of Mg supplementation on cardiovascular events and all-cause mortality. Mg supplementation is cheap and widely available, which would make this intervention accessible and affordable to all patients with CKD.

Contributors IB, DH, MS, CK and LB conceived the trial. IB wrote the initial draft. DH, MS, CK and LB provided feedback and comments. All authors approved the final version of the manuscript prior to submission. IB took responsibility for the submission process.

Funding This work is funded by Nordsjælland's Hospital's Research Foundation, The Danish Society of Nephrology's Research Foundation, Helen and Ejnar Bjørnow's Foundation, The Danish Kidney Foundation and The Toyota Foundation. Gunnar Kjems provided the study medication free of charge but has had no role in the design of the trial.

Competing interests None declared.

Ethics approval The Danish National Committees on Biomedical Research Ethics and the Norwegian National Committees on Biomedical Research Ethics.

Provenance and peer review Not commissioned; externally peer reviewed.

Open Access This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

© Article author(s) (or their employer(s) unless otherwise stated in the text of the article) 2017. All rights reserved. No commercial use is permitted unless otherwise expressly granted.

REFERENCES

1. Coresh J, Selvin E, Stevens LA, *et al.* Prevalence of chronic kidney disease in the United States. *JAMA* 2007;298:2038–47.
2. Go AS, Chertow GM, Fan D, *et al.* Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 2004;351:1296–305.

3. Budoff MJ, Rader DJ, Reilly MP, *et al.* Relationship of estimated GFR and coronary artery calcification in the CRIC (Chronic renal insufficiency cohort) Study. *Am J Kidney Dis* 2011;58:519–26.
4. Chirinos JA, Khan A, Bansal N, *et al.* Arterial stiffness, central pressures, and incident hospitalized heart failure in the chronic renal insufficiency cohort study. *Circ Heart Fail* 2014;7:709–16.
5. Bansal N, McCulloch CE, Lin F, *et al.* Different components of blood pressure are associated with increased risk of atherosclerotic cardiovascular disease versus heart failure in advanced chronic kidney disease. *Kidney Int* 2016;90:1348–56.
6. Matsushita K, Sang Y, Ballew SH, *et al.* Subclinical atherosclerosis measures for cardiovascular prediction in CKD. *J Am Soc Nephrol* 2015;26:439–47.
7. Yamada S, Giachelli CM. Vascular calcification in CKD-MBD: Roles for phosphate, FGF23, and Klotho. *Bone* 2016. Epub ahead of print: pii: S8756-3282(16)30345-3.
8. Montezano AC, Zimmerman D, Yusuf H, *et al.* Vascular smooth muscle cell differentiation to an osteogenic phenotype involves TRPM7 modulation by magnesium. *Hypertension* 2010;56:453–62.
9. Kircelli F, Peter ME, Sevinc Ok E, *et al.* Magnesium reduces calcification in bovine vascular smooth muscle cells in a dose-dependent manner. *Nephrol Dial Transplant* 2012;27:514–21.
10. Louvet L, Büchel J, Steppan S, *et al.* Magnesium prevents phosphate-induced calcification in human aortic vascular smooth muscle cells. *Nephrol Dial Transplant* 2013;28:869–78.
11. Montes de Oca A, Guerrero F, Martinez-Moreno JM, *et al.* Magnesium inhibits wnt/ β -catenin activity and reverses the osteogenic transformation of vascular smooth muscle cells. *PLoS One* 2014;9:e89525.
12. Xu J, Bai Y, Jin J, *et al.* Magnesium modulates the expression levels of calcification-associated factors to inhibit calcification in a time-dependent manner. *Exp Ther Med* 2015;9:1028–34.
13. Zelt JG, McCabe KM, Svajger B, *et al.* Magnesium modifies the impact of calcitriol treatment on vascular calcification in experimental chronic kidney disease. *J Pharmacol Exp Ther* 2015;355:451–62.
14. Sonou T, Ohya M, Yashiro M, *et al.* Magnesium prevents phosphate-induced vascular calcification via TRPM7 and Pit-1 in an aortic tissue culture model. *Hypertens Res* 2017. Epub ahead of print: 26 Jan 2017.
15. Alesutan I, Tuffaha R, Auer T, *et al.* Inhibition of osteo/chondrogenic transformation of vascular smooth muscle cells by MgCl₂ via calcium-sensing receptor. *J Hypertens* 2017;35:523–32.
16. Kanbay M, Yilmaz MI, Apetrii M, *et al.* Relationship between serum magnesium levels and cardiovascular events in chronic kidney disease patients. *Am J Nephrol* 2012;36:228–37.
17. Sakaguchi Y, Fujii N, Shoji T, *et al.* Hypomagnesemia is a significant predictor of cardiovascular and non-cardiovascular mortality in patients undergoing hemodialysis. *Kidney Int* 2014;85:174–81.
18. Sakaguchi Y, Fujii N, Shoji T, *et al.* Magnesium modifies the cardiovascular mortality risk associated with hyperphosphatemia in patients undergoing hemodialysis: a cohort study. *PLoS One* 2014;9:e116273.
19. Ishimura E, Okuno S, Yamakawa T, *et al.* Serum magnesium concentration is a significant predictor of mortality in maintenance hemodialysis patients. *Magnes Res* 2007;20:237–44.
20. João Matias P, Azevedo A, Laranjinha I, *et al.* Lower serum magnesium is associated with cardiovascular risk factors and mortality in haemodialysis patients. *Blood Purif* 2014;38(3-4):244–52.
21. Fein P, Weiss S, Ramos F, *et al.* Serum magnesium concentration is a significant predictor of mortality in peritoneal Dialysis patients. *Adv Perit Dial* 2014;30:90–3.
22. de Roij van Zijdewijn CL, Grooteman MP, Bots ML, *et al.* Serum magnesium and sudden death in European Hemodialysis Patients. *PLoS One* 2015;10:e0143104.
23. Lacson E, Wang W, Ma L, *et al.* Serum magnesium and mortality in Hemodialysis Patients in the United States: a Cohort Study. *Am J Kidney Dis* 2015;66:1056–66.
24. Kurita N, Akizawa T, Fukagawa M, *et al.* Contribution of dysregulated serum magnesium to mortality in hemodialysis patients with secondary hyperparathyroidism: a 3-year cohort study. *Clin Kidney J* 2015;8:744–52.
25. Tin A, Grams ME, Maruthur NM, *et al.* Results from the Atherosclerosis risk in communities study suggest that low serum magnesium is associated with incident kidney disease. *Kidney Int* 2015;87:820–7.
26. Van Laecke S, Nagler EV, Verbeke F, *et al.* Hypomagnesemia and the risk of death and GFR decline in chronic kidney disease. *Am J Med* 2013;126:825–31.
27. Sakaguchi Y, Iwatani H, Hamano T, *et al.* Magnesium modifies the association between serum phosphate and the risk of progression to end-stage kidney disease in patients with non-diabetic chronic kidney disease. *Kidney Int* 2015;88:833–42.
28. Sakaguchi Y, Hamano T, Nakano C, *et al.* Association between Density of coronary artery calcification and serum magnesium levels among patients with chronic kidney disease. *PLoS One* 2016;11:e0163673.
29. Turgut F, Kanbay M, Metin MR, *et al.* Magnesium supplementation helps to improve carotid intima media thickness in patients on hemodialysis. *Int Urol Nephrol* 2008;40:1075–82.
30. Mortazavi M, Moeinzadeh F, Saadatnia M, *et al.* Effect of magnesium supplementation on carotid intima-media thickness and flow-mediated dilatation among hemodialysis patients: a double-blind, randomized, placebo-controlled trial. *Eur Neurol* 2013;69:309–16.
31. Agatston AS, Janowitz WR, Hildner FJ, *et al.* Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol* 1990;15:827–32.
32. Pasch A, Farese S, Gräber S, *et al.* Nanoparticle-based test measures overall propensity for calcification in serum. *J Am Soc Nephrol* 2012;23:1744–52.
33. Russo D, Corrao S, Miranda I, *et al.* Progression of coronary artery calcification in predialysis patients. *Am J Nephrol* 2007;27:152–8.
34. Russo D, Miranda I, Ruocco C, *et al.* The progression of coronary artery calcification in predialysis patients on calcium carbonate or sevelamer. *Kidney Int* 2007;72:1255–61.
35. Bressendorff I, Hansen D, Schou M, *et al.* Oral magnesium supplementation in chronic kidney disease stages 3 and 4: efficacy, Safety, and effect on serum calcification Propensity—A Prospective Randomized Double-Blinded Placebo-Controlled Clinical Trial. *Kidney Int Rep* 2017;2:380–9.
36. Smith ER, Ford ML, Tomlinson LA, *et al.* Serum calcification propensity predicts all-cause mortality in predialysis CKD. *J Am Soc Nephrol* 2014;25:339–48.
37. Böhmer T, Røseth A, Holm H, *et al.* Bioavailability of oral magnesium supplementation in female students evaluated from elimination of magnesium in 24-hour urine. *Magnes Trace Elem* 1990;9:272–8.
38. Walker AF, Marakis G, Christie S, *et al.* Mg citrate found more bioavailable than other Mg preparations in a randomised, double-blind study. *Magnes Res* 2003;16:183–91.
39. de Francisco AL, Leidig M, Covic AC, *et al.* Evaluation of calcium acetate/magnesium carbonate as a phosphate binder compared with sevelamer hydrochloride in haemodialysis patients: a controlled randomized study (CALMAG study) assessing efficacy and tolerability. *Nephrol Dial Transplant* 2010;25:3707–17.
40. Block GA, Wheeler DC, Persky MS, *et al.* Effects of phosphate binders in moderate CKD. *J Am Soc Nephrol* 2012;23:1407–15.
41. Seifert ME, de las Fuentes L, Rothstein M, *et al.* Effects of phosphate binder therapy on vascular stiffness in early-stage chronic kidney disease. *Am J Nephrol* 2013;38:158–67.